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Flavonol and flavone intake and the risk of intermittent claudication in male smokers

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Abstract. The objective of this study was to investigate the association between flavonol and flavone intake and the risk of intermittent claudication in male smokers. The study population consisted of participants of the Finnish α-Tocopherol, β-Carotene Cancer Prevention (ATBC) Study, who were free of intermittent claudication at study entry. These 25,041 male smokers were 50–69 years old at baseline. Participants completed a validated dietary questionnaire at baseline. The occurrence of intermittent claudication was assessed by annual administration of the Rose questionnaire. During the median follow-up of 4.1 years, 2412 new cases of intermittent claudication were observed. Dietary intake of flavonols and flavones was inversely associated with the risk of intermittent claudication when adjusted for cardiovascular risk factors (relative risk, RR in the highest vs. lowest quintile of intake 0.86, 95% confidence interval, CI: 0.75–0.98, *p* for trend 0.007). However, after further adjustment for intakes of vitamins C and E and total carotenoids, the association was attenuated (RR: 0.93, 95% CI: 0.81–1.08, *p* for trend 0.12). The risk of intermittent claudication was lower among men in the highest quintile of vegetable consumption (RR: 0.78, 95% CI: 0.69–0.89, *p* for trend 0.0001) and among wine drinkers (RR: 0.63, 95% CI: 0.41–0.98). Adjustment for flavonol and flavone intake only marginally changed these associations. In conclusion, flavonol and flavone intake was not independently associated with the risk of intermittent claudication.

Key words: Antioxidants, Cohort studies, Flavonoids, Intermittent claudication

Abbreviations: CI = confidence interval; LDL = low-density lipoprotein; RR = relative risk

Introduction

Intermittent claudication is a condition in which walking induces pain in the calves that forces a cessation in activity. During rest, pain is alleviated but will reappear with resumed exertion. The pain is caused by insufficient blood flow to muscles due to atherosclerosis in the peripheral arteries. Free radicals are suggested to promote atherosclerosis through oxidation of low-density lipoprotein (LDL) that has several atherogenic properties [1]. Inhibition of LDL oxidation is hypothesized to prevent the development of atherosclerosis. Accordingly, dietary antioxidants are hypothesized to be protective.

Flavonols and flavones are polyphenols found ubiquitously in plants, especially in onions, tea leaves, and apples. *In vitro* studies have shown these to be effective antioxidants by scavenging free radicals, protecting vitamins C and E from oxidation, breaking oxidative chain reactions, and quenching the formation of singlet oxygen [2, 3]. Quercetin, the most studied compound among flavonols and flavones, has been demonstrated to inhibit macrophage-mediated LDL-oxidation [2, 4] and the formation of oxyradicals by inhibiting xanthine oxidase, and to protect cultured porcine endothelial cells against oxyradicals [5]. In human intervention studies, quercetin has had no effect on thrombogenic risk factors, blood pressure, or serum cholesterol levels [6–8]. Neither was quercetin identified in LDL or VLDL particles, although supplementation with quercetin markedly enhanced plasma quercetin concentrations [6].

The association between dietary intake of flavonols and flavones and the risk of cardiovascular diseases has not been consistent in previous epidemiologic studies. An inverse association has been observed in five studies [9–13] and no association in three studies [14–16]. To our knowledge the association between the intake of flavonols and flavones and the risk of intermittent claudication has not been evaluated earlier. However, the association between the intake of other antioxidants and the risk of peripheral arterial disease has been evaluated in few epidemiologic

studies. Vitamin C intake has been inversely associated with the risk in all of them [17–20]. In addition, in the α-Tocopherol, β-Carotene Cancer Prevention (ATBC) Study cohort dietary carotenoids and γ-tocopherol were inversely associated with the risk of intermittent claudication [19]. We examined here whether dietary intake of flavonols and flavones is independently associated with the risk of intermittent claudication in the ATBC Study cohort.

Subjects and methods

ATBC Study

The ATBC Study was a randomized, double-blind, placebo-controlled primary prevention trial undertaken to determine whether supplementation with α-tocopherol or β-carotene would reduce the incidence of lung cancer in male smokers. The rationale, design, and methods of the study as well as participant characteristics have been described in detail elsewhere [21].

The participants of the ATBC study were recruited from the total male population aged 50–69 years living in south-western Finland (n = 290,406). To be eligible, subjects had to be smokers of at least five cigarettes per day at study entry and to give written informed consent. Exclusion criteria included a history of cancer or another serious disease limiting long-term participation; use of supplements containing vitamin E (>20 mg/day), vitamin A (>20,000 IU/day), or β-carotene (>6 mg/day); and treatment with anticoagulant agents. The eligible men (n = 29,133) were randomized into one of four supplementation regimens: α-tocopherol alone (daily dose 50 mg), β-carotene alone (20 mg), combined α-tocopherol and β-carotene, or placebo. Trial follow-up continued for 5–8 years. The ATBC Study was approved by the institutional review boards of the National Public Health Institute, Helsinki, Finland, and the National Cancer Institute, Bethesda, Maryland, USA.

Baseline measurements

At baseline, the men completed a questionnaire on general background characteristics, and medical and smoking histories. They were interviewed on intermittent claudication with a structured Rose questionnaire [22]. Height, weight, and blood pressure were measured. A blood sample was drawn and serum was stored at −70 °C. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol levels were determined enzymatically (CHOD-PAP method, Boehringer Mannheim, Germany) [23, 24].

Dietary assessment

Diet was assessed at baseline using a self-administered, modified diet history method [25]. The questionnaire included 276 food items and mixed dishes. It was used with a picture booklet of 122 photographs of foods, each with 3–5 different portion sizes. The subject was asked to report the frequency of consumption and the usual portion size of foods during the previous 12 months. Frequencies were reported as the number of times per month, week, or day. At the first baseline visit, the questionnaire along with the picture booklet was given to the subject to be completed at home. At the second baseline visit 2 weeks later, the questionnaire was returned, reviewed, and completed together with a study nurse.

Food consumption data were computed into daily nutrient intake values based on the food composition database and related software of the National Public Health Institute. Flavonol and flavone contents in foods are based mainly on composition analyses done by Hertog and colleagues [26, 27]. The flavonol content of berries is, however, based on Finnish analyses [28]. Total flavonol and flavone intake was calculated as the sum of intakes of quercetin, kaempherol, myricetin, luteolin, and apigenin.

The dietary method was validated in a pilot study carried out on 190 men prior to the ATBC study [25]. The men completed the questionnaire and then kept food records for 24 days, spread over 6 months, as the reference method. They filled in the questionnaire again at the end of the study. For flavonols and flavones the energy-adjusted Pearson correlations between the first and the second questionnaire and food records were 0.59 and 0.66, respectively.

Ascertainment of end-points

The participants visited the local study centers three times per year. Study nurses interviewed them annually about any intermittent claudication using the Rose questionnaire [22]. The first occurrence of symptoms typical of intermittent claudication was defined as the end-point. These symptoms included pain in one or both calves induced upon exertion and relieved by a rest of no longer than 10 min.

Statistical analysis

Participants with poorly completed dietary questionnaire (n = 2023) and those who at baseline reported a history of intermittent claudication or symptoms typical of intermittent claudication based on the Rose questionnaire (n = 2071) were excluded. Thus, 25,039 men were included in the present analysis, and among them 2412 men reported symptoms typical of intermittent claudication during follow-up.

The follow-up period was calculated from the date of randomization until occurrence of intermittent claudication, death, or the last visit during which the subject was interviewed. Median follow-up time was 4.1 years. Intakes of flavonols and flavones and other antioxidants were log-transformed and then energy-

adjusted by the regression residual method [29]. Alcohol intake was not energy-adjusted. Men were grouped into quintiles or deciles of energy-adjusted intakes of flavonols and flavones. The proportional hazards model was used to estimate relative risks (RR) and 95% confidence intervals (CI) of intermittent claudication for different intake levels of flavonols and flavones. Adjustment was made first for age and supplementation group, although α-tocopherol and β-carotene supplementation did not show any effect on risk for intermittent claudication [30]. Secondly for cardiovascular risk factors (years of smoking, number of cigarettes daily, smoking cessation, systolic blood pressure, serum total and HDL-cholesterol, education, leisure-time physical activity, history of diabetes) and, thirdly for other dietary antioxidants (total carotenoids, vitamins C and E). Continuous background variables were grouped into tertiles, and dietary antioxidants into quintiles. Linearity of trend was obtained using the Wald test to treat median values of each quintile as continuous variables in the proportional hazards model. Interactions between flavonol and flavone intake and the supplementation groups were tested using likelihood ratio test. In food group analysis, consumption of vegetables, fruits, and berries was divided into quintiles, and wine and tea into two groups (less than one glass/cup per day or more). Proportional hazards models were first adjusted for age and supplementation group and then for cardiovascular risk factors.

Results

The baseline characteristics of cases and noncases of intermittent claudication are presented in Table 1. Subjects with intermittent claudication were older, had higher serum total cholesterol, were less educated, and had stopped smoking less often compared with noncases.

The mean intake of flavonols and flavones was 10.0 mg (SD 6.8 mg), of which 3.7 mg was derived from vegetables, 2.5 mg from tea, 1.5 mg from fruits, 1.0 mg from berries, 0.2 mg from alcoholic beverages, and 1.1 mg from other sources. In the lowest deciles of flavonol and flavone intake, the major source was vegetables, whereas in the two highest intake deciles flavonols and flavones were derived mainly from tea (Figure 1, panel A). Of the total intake of flavonols and flavones, quercetin comprised 85.1%,

Table 1. Background characteristics of cases and noncases of intermittent claudication

	Cases of intermittent claudication, N = 2412	No intermittent claudication, N = 22,627
Median of		
Age, years	58.2 (54.3–62.4)	56.7 (53.2–60.9)
Smoking, years	39 (33–43)	36 (30–41)
No. of cigarettes daily	20 (12–25)	20 (10–25)
Body mass index, kg/m^2	26.1 (24.0–28.8)	26.0 (23.7–28.5)
Serum total cholesterol, mmol/1	6.27 (5.57–7.07)	6.14(5.43–6.92)
Serum HDL-cholesterol, mmol/1	1.14(0.96–1.36)	1.18 (1.00–1.42)
Systolic blood pressure, mm Hg	144 (130–160)	140(128–152)
Diastolic blood pressure, mm Hg	88 (80–96)	88 (80–94)
Percentage of group with		
Education (>11 years)	7.0	11.2
Leisure-time physical activity (⩾3 times per week)	17.3	19.6
Stopped smoking	10.5[a]	15.2
Wine drinkers [⩾1 glass (120 ml)/day]	0.9	1.7
Tea drinkers [⩾1 cup (170 ml/day]	9.4	9.7
Median daily intake of		
Flavonols and flavones, mg	7.67(5.28–11.54)	8.11 (5.54–12.23)
Energy, kcal	2720 (2230–3250)	2730 (2270–3270)
Alcohol, g	10.7 (2.7–24.8)	11.2(2.6–25.9)
Total carotenoids, mg	3.83 (2.72–5.48)	4.06 (2.89–5.72)
Vitamin C, mg	85.5(63.0–115.6)	89.0(65.1–119.5)
Vitamin E, mg	10.6 (7.9–14.3)	10.8 (8.2–14.6)
Median daily consumption of		
Vegetables, g	90.1 (54.5–136.5)	95.7 (59.9–144.3)
Fruits, g	67.8 (30.2–120.0)	71.4(32.6–121.5)
Berries, g	25.4(11.3–48.6)	26.5(11.9–49.3)

Medians with interquartile ranges or proportions are shown.
[a] Proportion of those who stopped smoking before intermittent claudication.

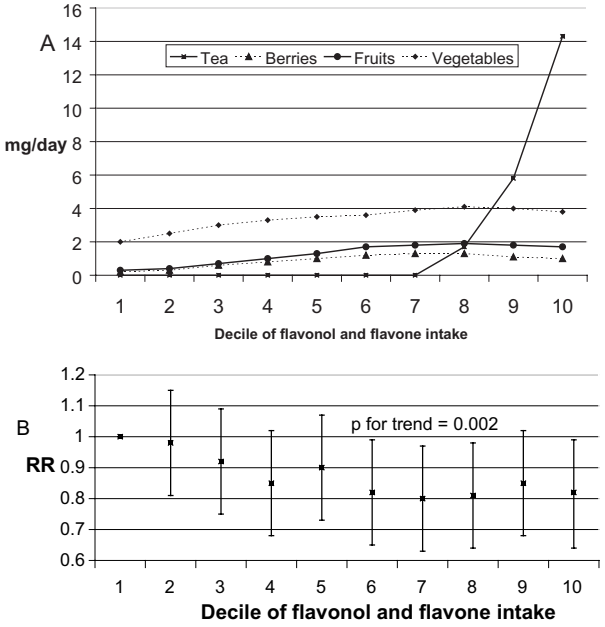

Figure 1. Panel A. Intake of flavonol and flavone from different sources in deciles of total intake. Panel B. RR and 95% CI of intermittent claudication adjusted for cardiovascular risk factors by decile of flavonol and flavone intake.

kaempherol 5.7%, myricetin 8.4%, luteolin 0.3%, and apigenin 0.5%. Flavonol and flavone intake correlated with intakes of vitamin C ($r = 0.50$) and total carotenoids ($r = 0.41$).

Intake of flavonols and flavones was inversely associated with the risk of intermittent claudication after adjustment for cardiovascular risk factors (Table 2). The relative risk declined with an increasing daily intake of up 7th decile; after that no additional decrease in risk was apparent (Figure 1, panel B). After further adjustment for intake of vitamins C and E and total carotenoids, the association markedly attenuated and was no longer significant (Table 2). Adjustment for vitamin C alone or total carotenoids alone attenuated the observed association, but adjustment for vitamin E alone showed no such effect. Neither α-tocopherol ($p = 0.23$) nor β-carotene ($p = 0.26$) supplementation showed an interaction with flavonol and flavone intake.

In the food group analysis, consumption of vegetables was inversely associated with the risk of intermittent claudication (multivariate RR in the highest vs. lowest quintile of intake 0.78, 95% CI: 0.69–0.89, p for trend <0.001, Table 3). Consumption of fruits and berries was inversely associated with the risk of intermittent claudication in age-adjusted models, but after further adjustment for cardiovascular risk factors, the associations became non-significant. No association was found between tea drinking and the risk of intermittent claudication. Those who drank at least one glass of wine per day had lower risk than those who drank less (RR: 0.63, 95% CI: 0.41–0.98). When vegetable consumption and wine drinking were simultaneously added to the model, the relative risks of vegetables (RR: 0.78, 95% CI: 0.69–0.89, p for trend <0.001) and wine (RR: 0.64, 95% CI: 0.42–0.99) were only slightly altered. Similarly, when either vegetable consumption or wine drinking was adjusted for intake of flavonols and flavones, the relative risks of both vegetable consumption (RR: 0.81, 95% CI: 0.71–0.94, p for trend 0.004) and wine drinking (RR: 0.66, 95% CI: 0.43–1.02) were for the most part unchanged. The inverse association of vegetable consumption also persisted after adjustment for flavonols and flavones derived from vegetables (RR: 0.82, 95% CI: 0.71–0.95). After further adjustment for total alcohol intake, the relation between wine drinking and the risk remained virtually unchanged (RR: 0.62, 95% CI: 0.40–0.96).

Discussion

In this cohort of male smokers, intake of flavonols and flavones was inversely associated with the risk of intermittent claudication, but this association was attenuated after adjustment for other dietary antioxidants, especially vitamin C and carotenoids. In addition, high consumption of vegetables and wine

Table 2. Intake of flavonols and flavones and the relative risk (95% CIs) of intermittent claudication

Quintile of intake	Median intake	Number of cases	Age-adjusted[a]	+ Cardiovascular risk factors[b]	+ Other antioxidants[c]
1st	4.0	547	1.00	1.00	1.00
2nd	6.1	502	0.87 (0.77–0.98)	0.92 (0.82–1.04)	0.97 (0.85–1.10)
3rd	8.1	490	0.84 (0.74–0.94)	0.89 (0.78–1.00)	0.95 (0.83–1.09)
4th	10.9	434	0.74 (0.65–0.84)	0.84 (0.74–0.96)	0.89 (0.77–1.03)
5th	18.0	439	0.73 (0.64–0.83)	0.86 (0.75–0.98)	0.93 (0.81–1.08)
p for trend			0.0001	0.007	0.12

[a] Adjusted for age and supplementation group.
[b] Adjusted for age, supplementation group, years of smoking, number of cigarettes per day, smoking cessation, systolic blood pressure, serum total cholesterol, HDL cholesterol, education, leisure-time physical activity, and history of diabetes.
[c] Additionally adjusted for intake of vitamins C and E and total carotenoids.

Table 3. Consumption of foods rich in flavonols and flavones and the RR (95% CIs) of intermittent claudication

	Number of cases	Age-adjusted RR[a]	Multivariate RR[b]
Vegetables[c]			
<52 g/day	561	1.00	1.00
52–80 g/day	481	0.81 (0.72–0.92)	0.84 (0.75–0.96)
81–111 g/day	501	0.83 (0.73–0.93)	0.87 (0.77–0.98)
112–158 g/day	448	0.73 (0.64–0.82)	0.79 (0.70–0.89)
>158 g/day	421	0.68 (0.60–0.77)	0.78 (0.69–0.89)
p for trend		0.0001	0.0001
Fruits[c]			
<26 g/day	523	1.00	1.00
26–55 g/day	497	0.90 (0.80–1.02)	0.94 (0.83–1.06)
56–89 g/day	444	0.79 (0.70–0.90)	0.83 (0.73–0.95)
90–136 g/day	493	0.88 (0.78–0.99)	0.94 (0.83–1.06)
>136 g/day	455	0.82 (0.72–0.93)	0.90 (0.79–1.02)
p for trend		0.003	0.13
Berries[c]			
<9 g/day	498	1.00	1.00
9–20 g/day	509	0.98 (0.86–1.10)	1.01 (0.89–1.15)
21–34 g/day	455	0.85 (0.75–0.96)	0.91 (0.80–1.04)
35–57 g/day	474	0.87 (0.77–0.99)	0.94 (0.83–1.07)
>57 g/day	476	0.85 (0.75–0.96)	0.94 (0.83–1.07)
p for trend		0.002	0.20
Tea			
<1 cup (170 ml)/day	2180	1.00	1.00
⩾cup/day	232	0.98 (0.86–1.13)	1.07 (0.94–1.23)
Wine			
<1 glass (120 ml)/day	2391	1.00	1.00
⩾1 glass/day	21	0.53 (0.35–0.82)	0.63 (0.41–0.98)

[a] Adjusted for age and supplementation group.
[b] Adjusted for age, supplementation group, years of smoking, number of cigarettes per day, smoking cessation, systolic blood pressure, serum total and HDL cholesterol, education, leisure-time physical activity, and history of diabetes.
[c] Classification based on quintiles.

drinking were related to lower risk of intermittent claudication.

A strength of our study was the large number of cases. This enabled us to study the association between the intake of flavonols and flavones and the risk of intermittent claudication with narrow intake intervals (deciles), and therefore, to investigate the dose-response more closely. Some attenuation of the association may exist due to the limitations in endpoint assessment. Intermittent claudication is a symptom that reflects peripheral atherosclerosis. Symptoms often fluctuate and spontaneous recovery is typical for it. We assessed intermittent claudication once a year by a questionnaire designed for large epidemiologic surveys. The sensitivity and specificity of the questionnaire has varied between 60–92% and 91–100%, respectively [22, 31]. Our incidence rate appeared to be somewhat higher than that of other studies [32] but the differences in age and smoking histories made the comparison difficult.

Misclassification in exposure, i.e. intake of flavonols and flavones, probably also occured. Part of this misclassification was due to calculation of intake estimates. The food frequency questionnaire was not designed to measure the intake of flavonols and flavones, e.g. red and white wines were not handled separately in the questionnaire. Measurement difficulties of foods containing flavonols and flavones further complicated classification. This was reflected in correlations between flavonol and flavone intakes based on food frequency questionnaires and food records being modest.

We observed an inverse association between the consumption of vegetables and the risk of intermittent claudication. High consumption of vegetables has been related to lower risk of other cardiovascular diseases, both in this cohort [13, 16] and in several other cohort studies [33–35]. Factors behind this association are, however, not very well known. The inverse association between vegetable consumption and the risk of intermittent claudication cannot be explained by flavonols and flavones, since relative risks of vegetable consumption were unchanged after adjustment for flavonol and flavone intake. Besides antioxidants and other nutrients (e.g. magnesium, potassium, dietary fiber) from vegetables, residual confounding of lifestyle factors that were not measured may explain part the observed inverse association.

Risk of intermittent claudication was smaller among wine drinkers than nondrinkers. Wine drink-

ing was also inversely associated with the risk of coronary heart disease in this same cohort [13]. Adding the intake of flavonols and flavones or total alcohol intake to the multivariate model did not substantially alter the association between wine drinking and the risk of intermittent claudication. One should note that there are other flavonoids in wine that could have beneficial properties against intermittent claudication – a glass of wine does contain over 100 mg of polyphenolic compounds [36]. Another explanation for the observed inverse association is other lifestyle factors. In this cohort, for instance, wine drinkers were more educated than others. Although many of these background factors were taken into account, some residual confounder effects probably remained. Furthermore, only 1.6% of the participants drank at least one glass of wine per day. Thus, the inverse association was based on a small number of cases ($n = 21$), and one must bear in mind the possibility of a chance finding.

Our study only contained smokers. Since smoking interferes in human oxidative processes and is a major risk factor for atherosclerosis, flavonol and flavone intake could be differently associated with the risk of intermittent claudication in smokers and nonsmokers. Our results, therefore, cannot be generalized to nonsmokers.

In conclusion, high intake of flavonols and flavones was inversely associated with the risk of intermittent claudication in a cohort of male smokers. The association was, however, markedly attenuated and became nonsignificant after adjustment for other dietary antioxidants. High consumption of vegetables was related to lower risk, which was not attenuated after adjustment for their flavonol and flavone content. Thus, dietary flavonols and flavones have no independent effect on the manifestation of intermittent claudication.

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References

1. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet 1994; 344: 793–795.
2. De Whalley CV, Rankin SM, Hoult JRS, Jessup W, Leake DS. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. Biochem Pharmacol 1990; 39: 1743–1750.
3. Kandaswami C, Middleton E. Free radical scavenging and antioxidant activity of plant flavonoids. Adv Exp Med Biol 1994; 366: 351–376.
4. Aviram M, Fuhrman B. Polyphenolic flavonoids inhibit macrophage-mediated oxidation of LDL and attenuate atherogenesis. Atherosclerosis 1998; 137: S45–S50.
5. Zeng LH, Wu J, Fung B, Tong JH, Mickle D, Wu TW. Comparative protection against oxyradicals by three flavonoids on cultured endothelial cells. Biochem Cell Biol 1997; 75: 717–720.
6. Conquer JA, Maiani G, Azzini E, Raguzzini A, Holub BJ. Supplementation with quercetin markedly increases plasma quercetin concentration without effect on selected risk factors for heart disease in healthy subjects. J Nutr 1998; 128: 593–597.
7. Jansen KPLTM, Mensink RP, Cox FJJ, et al. Effects of the flavonoids quercetin and apigenin on hemostasis in healthy volunteers: results from an in vitro and a dietary supplement study. Am J Clin Nutr 1998; 67: 255–262.
8. McAnlis GT, McEneny J, Pearce J, Young IS. Absorption and antioxidant effects of quercetin from onions, in man. Eur J Clin Nutr 1999; 53: 92–96.
9. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of heart disease: the Zutphen Elderly Study. Lancet 1993; 342: 1007–1011.
10. Knekt P, Järvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: A cohort study. Br Med J 1996; 312: 478–481.
11. Rimm EB, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. Ann Intern Med 1996; 125: 384–389.
12. Yochum L, Kushi LH, Meyer K, Folsom AR. Dietary flavonoid intake and risk of cardiovascular disease in postmenopausal women. Am J Epidemiol 1999; 149: 943–949.
13. Hirvonen T, Pietinen P, Virtanen M, et al. Intake of flavonols and flavones and risk of coronary heart disease in male smokers. Epidemiology 2001; 12: 62–67.
14. Keli SO, Hertog MGL, Feskens EJM, Kromhout D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke. Arch Intern Med 1996; 156: 637–642.
15. Hertog MGL, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D. Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly Study. Am J Clin Nutr 1997; 65: 1489–1494.
16. Hirvonen T, Virtamo J, Korhonen P, Albanes D, Pietinen P. Intake of flavonoids, carotenoids, vitamins C and E, and risk of stroke in male smokers. Stroke 2000; 31: 2301–2306.
17. Donnan PT, Thomson M, Fowkes FGR, Prescott RJ, Housley E. Diet as risk factor for peripheral arterial disease in the general population: The Edinburgh Artery Study. Am J Clin Nutr 1993; 57: 917–921.
18. Katsoyanni K, Skalkidis Y, Petridou E, Polychronopolou-Trichopolou A, Willett W, Trichopoulos D. Diet and peripheral arterial occlusive disease: The role of poly-, mono-, and saturated fatty acids. Am J Epidemiol 1991; 133: 24–31.
19. Törnwall ME, Virtamo J, Haukka JK, Aro A, Albanes D, Huttunen J. Prospective study of diet, lifestyle, and intermittent claudication in male smokers. Am J Epidemiol 2000; 151: 892–901.

20. Klipstein-Grobusch K, den Breeijen JH, Grobbee DE, Boeing H, Hofman A, Witteman JCM. Dietary antioxidants and peripheral arterial disease. Am J Epidemiol 2001; 154: 145–149.
21. The ATBC Cancer Prevention Study Group. The α-Tocopherol, β-Carotene Lung Cancer Study: Design, methods, participant characteristics, and compliance. Ann Epidemiol 1994; 4: 1–10.
22. Rose GA. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bull World Health Organ 1962; 27: 645–658.
23. Kattermann R, Jaworek D, Möller G, et al. Multicentre study of a new enzymatic method of cholesterol determination. J Clin Chem Clin Biochem 1984; 22: 245–251.
24. Kostner GM. Enzymatic determination of cholesterol in high-density lipoprotein fractions prepared by polyanion precipitation. Clin Chem 1976; 22: 695.
25. Pietinen L, Hartman AM, Haapa E, et al. Reproducibility and validity of dietary assessment instruments, I: A self-administered food use questionnaire with a portion size picture booklet. Am J Epidemiol 1988; 128: 655–666.
26. Hertog MGL, Hollman PC, Katan MB. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J Agric Food Chem 1992; 40: 2379–2383.
27. Hertog MGL, Hollman PC, van de Putte P. Content of potentially anticarcinogenic flavonoids in tea infusions, wines, and fruit juices. J Agric Food Chem 1993; 41: 1242–1246.
28. Häkkinen SH, Kärenlampi SO, Heinonen IM, Mykkänen HM, Törrönen AR. Content of the flavonols quercetin, myricetin and kaempherol in 25 edible berries. J Agric Food Chem 1999; 47: 2274–2279.
29. Willett WC, Stampfer MJ. Total energy intake – implications for epidemiologic analyses. Am J Epidemiol 1986; 124: 17–27.
30. Törnwall ME, Virtamo J, Haukka JK, Aro A, Albanes D, Huttunen JK. The effect of alpha-tocopherol (vitamin E) and beta-carotene supplementation on the incidence of intermittent claudication in male smokers. Arterioscler Thromb Vasc Biol 1997; 17: 3475–3480.
31. Leng GC, Fowkes FGR. The Edinburg claudication questionnaire: An improved version of the WHO/Rose questionnaire for use in epidemiologic surveys. J Clin Epidemiol 1992; 45: 1101–1109.
32. Bowlin SJ, Medalie JH, Flocke SA, Zyzanski SJ, Goldbourt U. Epidemiology of intermittent claudiaction in middle-aged men. Am J Epidemiol 1994; 140: 418–430.
33. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. N Engl J Med 1993; 328: 1450–1456.
34. Knekt P, Reunanen A, Järvinen R, Seppänen R, Heliövaara M, Aromaa A. Antioxidant vitamin intake and coronary mortality in a longitudinal study. Am J Epidemiol 1994; 139: 180–189.
35. Pandey DK, Shekelle R, Selwyn BJ, Tangney C, Stamler J. Dietary vitamin C and beta-carotene and risk of death in middle-aged men: The Western Electric study. Am J Epidemiol 1995; 142: 1269–1278.
36. Bravo L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 1998; 56: 317–333.

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